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Transplant-Associated Microangiopathy in Patients Receiving Tacrolimus Following Allogeneic Stem Cell Transplantation: Risk Factors and Response to Treatment

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ABSTRACT

Transplant-associated microangiopathy (TAM) is a life-threatening complication after allogeneic HSCT, particularly with the use of calcineurin inhibitors as post-transplantation immunosuppressive therapy. We report our experience with TAM after HSCT with tacrolimus-based GVHD prophylaxis in a single-center study. Sixty-six of 1219 transplant recipients developed TAM with a cumulative incidence of 5.9%. Risk factors for TAM were female gender, lymphoid malignancy, receipt of a matched unrelated donor, and grade II-IV aGVHD. Most patients had infection and/or active GVHD at the diagnosis of TAM (82%). In the absence of renal dysfunction or encephalopathy, tacrolimus was generally continued, maintaining blood levels within the lower therapeutic range. Sixty-three patients were treated with plasma exchange. The cumulative incidence of response of TAM was 60%. Only 1 patient had a response of TAM without resolution of concomitant infections or GVHD. Six-month survivals were 0% and 50% for TAM nonresponders and responders, respectively. In conclusion, TAM is a common, life-threatening complication of allogeneic hematopoietic transplantation using tacrolimus prophylaxis. Control of TAM generally requires response of associated infections and GVHD. TMA response may occur despite continuation of tacrolimus treatment.

KEY WORDS

Transplant-associated microangiopathy • Graft-versus-host disease • Tacrolimus

INTRODUCTION

Transplant-associated microangiopathy (TAM) is one of the most serious complications after allogeneic HSCT [1]. Clinical manifestations include severe thrombocytopenia, microangiopathic hemolysis, and frequently renal dysfunction and neurologic complications. The reported incidence of TAM after HSCT varies from 0.5% to 70%, indicating the inconsistencies in the criteria for diagnosis in the literature and variability in patient, disease, and treatment characteristics of the study groups [2-4].

Diffuse endothelial cell injury; tissue factor expression, exposure of the thrombogenic subendothelial matrix, and in turn platelet activation and aggregation are considered the basis of the pathophysiology of TAM [5-8]. Transplantation from an unrelated or mismatched donor, use of calcineurin inhibitors, and the presence of infection and GVHD have been reported as risk factors for development of TAM after allogeneic HSCT [3,9-12].

There is no consensus for treatment of post-transplantation TAM. In contrast to idiopathic thrombotic thrombocytopenic purpura, TAM is not associated with a marked decrease in ADAMTS13 (von Willebrand factor cleaving protease) level or its inhibitors [13-15]. The efficacy of therapeutic plasma exchange (PE) has not been established for TAM. Cyclosporine-

induced TAM may respond to dose reduction or discontinuation [9,16], but the literature for management of tacrolimus-induced TAM is less informative. TAM remains a life-threatening complication after allogeneic HSCT [1,2].

We report the risk factors for development of TAM after allogeneic HSCT for hematologic malignancies with tacrolimus-based GVHD prophylaxis and the factors associated with a favorable treatment response with therapeutic PE.

METHODS

Patient Eligibility and Data Collection

All adult patients receiving first allogeneic BMT or blood progenitor cell transplantation as treatment for hematologic malignancies and myelodysplastic syndrome at the University of Texas/MD Anderson Cancer Center from January 1, 1998 through December 31, 2004 were reviewed. In the analysis were included 1219 consecutive patients who had engraftment after non-T cell-depleted transplantation and tacrolimus-based GVHD prophylaxis. Retrospective chart review was reviewed and approved by the MD Anderson Cancer Center institutional review board. Data on patient, donor, and disease characteristics, treatment, and outcomes of transplantation were collected.

Conditioning Regimens and GVHD Prophylaxis

Different preparative regimens were used. We grouped preparative regimens as myeloablative and reduced intensity conditioning (RIC) using the criteria of the Center for International Blood and Marrow Transplantation Research. Five hundred seventy-one patients (46.8%) were treated with ablative regimens that primarily included busulfan and cyclophosphamide (n = 193), cyclophosphamide and >10 Gy TBI (n = 152), fludarabine and high-dose busulfan (n = 119), and carmustine, etoposide, cytarabine, and melphalan (n = 67). Six hundred forty-eight patients (53.3%) received RIC mostly including fludarabine and melphalan (n = 239) or fludarabine and busulfan with a busulfan dose <12 mg/kg (n = 94) or fludarabine/cyclophosphamide with or without rituximab (n = 193).

Donor BM or blood progenitor cells were procured using standard mobilization and harvesting techniques. These were infused i.v. on day 0. For ABO incompatible BM transplants, erythrocytes or plasma were depleted as indicated to prevent hemolysis. BM or PB progenitor cells procured from unrelated donors were obtained through National Marrow Donor Program.

The transplant was from an HLA-identical related donor in 662 cases (54.3%) and from an unrelated donor in 448 cases (36.8%). At least 1 antigen mismatched related and unrelated donor, by serologic class I and high-resolution molecular DRB1 typing, was used in 77 (6.3%) and 32 (2.6%) cases, respectively. The cell source was BM in 594 (48.7%), PB progenitor cells in 602 (49.4%), and cord blood in 23 (1.9%).

GVHD prophylaxis was tacrolimus based in all patients; 1197 patients (98.2%) received methotrexate 5 mg/m^2 i.v. on days 1, 3, 6, and, in some patients, 11 after transplantation. ATG was given to 299 patients (24.5%), primarily those receiving HLA nonidentical or unrelated donor transplants. Tacrolimus was started on day −2 and dosing was adjusted to maintain blood levels of 5 to 15 ng/dL during the first 3 to 6 mo and then tapered depending on the protocol and the presence or absence of GVHD. No patients received sirolimus or T cell depletion for GVHD prophylaxis.

Definitions

Our definition of TAM was based on the recent diagnostic criteria proposed by an international working group [17]. The diagnosis of TAM after allogeneic HSCT required fulfillment of the following criteria: (a) schistocytosis ≥1 per high-power field on blood smear film; (b) de novo, prolonged, or progressive thrombocytopenia (platelet count <50 000 or ≥50% decrease from baseline values); (c) sudden or persistent increase ≥50% in lactate dehydrogenase (LDH) compared with baseline value; (d) decrease in hemoglobin concentration or increased RBC transfusion requirement; (e) if available, decrease in serum haptoglobin level; (f) negative Coombs test results; and (g) normal prothrombin time and activated partial thromboplastin time.

Response to therapy for TAM required fulfillment of the following criteria: (a) none or only occasional schistocytes per high-power field on blood smear film; (b) >50% decrease in LDH, which was sustained for ≥7 d without PE; (c) no evidence of ongoing hemolysis, including normal indirect bilirubin and serum haptoglobin levels when available; and (d) resolution of nonfocal neurologic symptoms.

The indication for transplantation was categorized as myeloid malignancies including AML, myelodysplastic syndrome, and CML, or lymphoid malignancies including ALL, CLL, multiple myeloma, and lymphomas. Disease status at allogeneic HSCT was defined according to the following criteria: "good-risk" disease status included acute leukemias with marrow aspirate containing <5% blasts and recovering PB counts, CML in chronic phase, multiple myeloma, and lymphomas in remission. Other patients with more advanced diseases were considered to have "poor-risk" disease status.

Day 0 was the stem cell infusion day. The diagnosis of acute and chronic GVHD was based on consensus criteria [18,19]. Status of GVHD at time of TAM diagnosis was categorized as no, quiescent, or active GVHD. GVHD was considered "quiescent" if the clinical manifestations had resolved with treatment and the immunosuppressive treatment was being tapered.

CMV infection/reactivation was defined as a positive culture or pp65 antigenemia assay based on ≥1 $pp65^+$ cell/10^6 neutrophils assessed. Invasive fungal infection required evidence of infection by biopsy or the isolation of fungus from typically sterile specimens. Based on these definitions, infectious diseases at TAM were categorized as (a) isolated CMV infection, (b) fungal infection with or without viral or bacterial infections, or (c) viral and/or bacterial infections. Infections that were diagnosed within the 14 d before diagnosis of TAM and were active at the diagnosis of TAM were included.

Management of TAM

Tacrolimus was generally continued with dose adjustment into the lower therapeutic range (5-10 ng/mL) and was permanently discontinued only for progressive renal failure or encephalopathy. Corticosteroids were used for treatment of GVHD and were routinely initiated or increased for prevention of GVHD if tacrolimus was discontinued. Concomitant infections were treated with antimicrobial therapy based on results of culture and sensitivity testing.

Therapeutic PE was performed using a Cobe Spectra cell separator (Gambro BCT, Lakewood, Co). Patients provided written informed consent for the procedure. One time to 1.5 times the plasma volume was exchanged with a mixture of albumin with fresh frozen plasma or cryosupernatant plasma. PE was continued until a clinical response was documented or patients' clinical condition deteriorated such that they were considered to have treatment failure. The frequency of PE was typically daily at the beginning and titrated as needed to control disease manifestations to several times weekly and then once weekly depending on response. PE was discontinued when criteria for response were achieved without exacerbation between apheresis sessions. Platelet transfusions were administered when the platelet count was $<20 \times 10^9$/L or in the presence of major bleeding.

Statistical Methods

The cumulative incidence of TAM and response to PE were analyzed as death without TAM and death without response to therapy as competing risks, respectively [20]. Risk factors for TAM development and predictors of response to TAM were evaluated using Cox proportional hazards model. Risk factors analyzed for TAM included patient's age (as quartiles), gender, disease diagnosis (myeloid versus lymphoid), donor type (matched related donor, matched unrelated donor, mismatched related donor, mismatch unrelated donor), disease status at HSCT (good risk versus poor risk disease status), use of TBI in the preparative regimen, intensity of preparative regimen (myeloablative versus RIC), stem cell source (BM, blood pressure, cord blood), and occurrence of grade II-IV aGVHD as a time-dependent variable. Patients who received a second allogeneic HSC transplant had follow-up time censored at the time of second stem cell infusion. Predictive factors for response included disease characteristics of TAM including renal dysfunction, neurologic dysfunction, GVHD (none, quiescent, active), infection (isolated CMV infection, fungal infection with or without bacterial and/or viral infections, bacterial and/or viral infection), and observation of supratherapeutic blood levels of tacrolimus. In Cox proportional hazards models, variables that were significant at the .1 level on univariate analysis or were considered to be clinically significant were evaluated in a multivariate analysis. For response evaluation, only patients with their first episode of TAM were included. Overall survival was estimated by Kaplan-Meier curves and comparisons were preformed by log-rank test. Comparisons of pre- and post-TAM tacrolimus levels were performed using Wilcoxon tests. Statistical significance was determined at the .05 level. All *P* values were 2-sided. Statistical analysis was performed using STATA 7.0, SAS 9.1 (SAS Institute Inc., Cary, NC), and S-plus 6.0.

RESULTS

Incidence and Time of Onset of TAM

Median follow-up time for surviving patients was 27.4 mo after SCT (range, 1.2-88 mo). Sixty-six of 1219 patients developed TAM, with a cumulative incidence of 5.9%. Median interval from transplantation to TAM was 67 d (range, 11 d-5 yr), with only 4 patients presenting after a year after transplantation. Onset of TAM was within 100 d after transplantation in 45 patients (68.2%) and after day 100 in 21.

Risk Factors for Development of TAM

Patient characteristics and their association with TAM are presented in Table 1. On univariate analysis, significant risk factors associated with development of TAM were gender, donor type, and aGVHD grade II-IV. Cumulative incidences of TAM with each of the risk factors are summarized in Figures 1-3. In multivariate analysis by Cox proportional hazards model, results were similar to those obtained in univariate analysis with the exception that the effect of lymphoid malignancy diagnosis, which was marginally

Table 1. *Patient Characteristics and Their Association with TAM by Univariate Cox Proportional Hazards Models*

	No. of Patients (%)	HR	95% CI	P
Age (yr)				
<47	621 (50.9)	1.00		
≥47	598 (49.1)	1.37	0.84-2.22	0.21
Gender				
Male	739 (60.6)	1.00		
Female	480 (39.4)	2.03	1.25-3.31	0.004
Diagnosis				
Myeloid malignancy	638 (52.3)	1.00		
Lymphoid malignancy	581 (47.7)	1.55	0.95-2.55	0.08
Disease status				
Good risk	451 (37)	1.00		
Poor risk	768 (63)	1.32	0.79-2.20	0.30
Donor type				
Matched related donor	662 (54.3)	1.00		
Matched unrelated donor	448 (36.8)	3.04	1.81-5.12	<0.001
Mismatched related donor	77 (6.3)	1.97	0.68-5.68	0.21
Mismatched unrelated donor	33 (2.6)	2.41	0.56-10.36	0.24
Preparative regimen				
Reduced intensity	648 (53.2)	1.00		
Myeloablative	571 (46.8)	1.02	0.63-1.66	0.93
TBI in preparative regimen				
No	1044 (85.6)	1.00		
Yes	175 (14.4)	1.56	0.85-2.86	0.15
aGVHD II-IV*		4.47	2.76-7.24	<0.001

HR indicates hazard ratio; CI, confidence interval.
*Time-dependent variable.

significant in univariate analysis (hazard ratio, 1.55; $P = .08$), became significant (hazard ratio, 1.75; $P = .03$; Table 2). Patient age, disease status, and intensity of preparative regimens were not associated with the risk of TAM in this study.

Clinical Features at Diagnosis of TAM

Of the 66 patients who developed TAM, 3 had 2 episodes. The clinical features of the 69 TAM episodes are summarized in Table 3. Median platelet count at diagnosis of TAM was 19×10^9/L (range, 5-47). In 35 episodes (50.7%), patients had de novo thrombocytopenia; in 34 episodes (49.3%), sustained thrombocytopenia was observed. Median LDH level was 2807 IU/L (upper limit of normal is 618 IU/L) and the median difference compared with baseline levels was 1785 IU/L (range, 578-

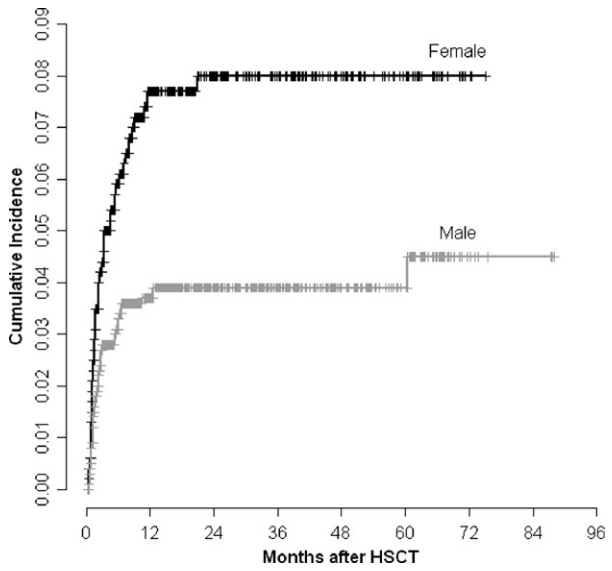

Figure 1. Cumulative incidences of TAM were 7.7% for female patients and 3.7% for male patients ($P = .003$).

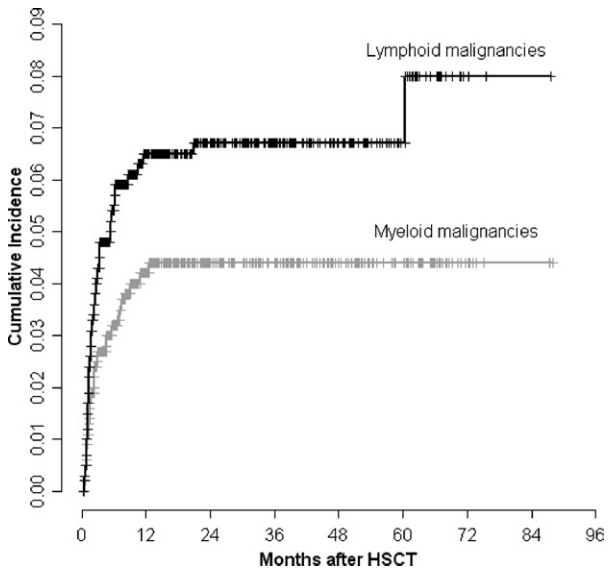

Figure 2. Cumulative incidences of TAM were 6.5% for patients with lymphoid malignancies and 4.2% for patients with myeloid malignancies ($P = .03$).

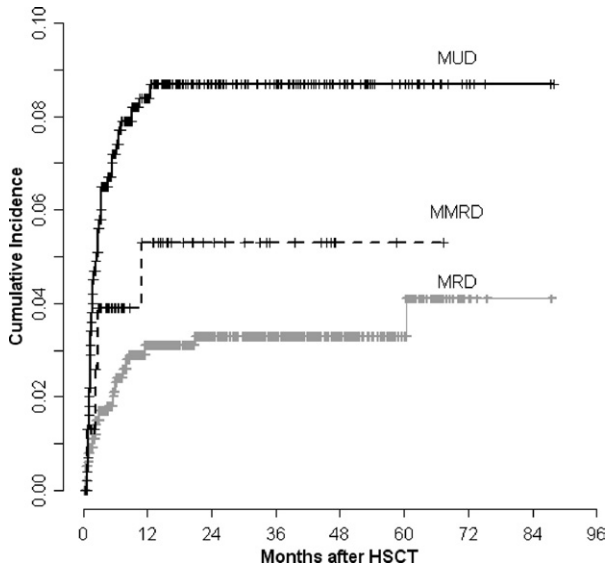

Figure 3. Compared with mismatched unrelated donor (MUD), cumulative incidence of TAM was lower for matched related donor (MRD) during the first 100 d after transplantation; this excessive incidence with MUD was also evident after day 100. Cumulative incidences of TAM were 3.1% for MRD, 8.4% for MUD, and 5.3% for mismatched related donor (MMRD; $P < .001$). Cumulative incidence of TAM for MMUD was not analyzed because of a small sample with few events.

9400 IU/L). Serum haptoglobin levels were available in 63 episodes and all were lower than our laboratory's maximum normal level of 30 mg/dL, and in 59 of 63 episodes it was <6 mg/dL. More than a 50% increase in serum total bilirubin was documented in 46 episodes (66.7%). An increase in indirect bilirubin could not be analyzed because only 10 patients had baseline levels available.

In 46 episodes (66.6%), patients were documented to have an active infection at the time of TAM diagnosis. Ten patients had isolated CMV, 22 had bacterial and/or other viral infection, and 14 had fungal infections. Two more patients were diagnosed with fungal infections during the course of TAM.

Renal function abnormality defined as ≥50% increase of baseline serum creatinine level occurred in 22 patients (32%) and neurologic dysfunction was observed in 28 cases (40%), respectively. The most common presentation of CNS involvement was mental status changes and/or headache.

Hypertension was common; systolic blood pressure >140 mm Hg was seen in 66 episodes (95%) with a median of 165 (range, 140-202) and diastolic blood pressure was >85 mm Hg in 57 episodes (83%) with a median of 100 (range, 85-120) at least once during the disease course despite appropriate treatment. Hemodialysis was required for renal failure in 22 episodes (32%) during the course of TAM.

Immunosuppressive Therapy

Patients were receiving systemic corticosteroid treatment in 62 of 69 episodes (90%) at the time of diagnosis of TAM; 29 for active GVHD, 24 for quiescent GVHD, and 9 for other reasons. In 35 episodes (56%), the methylprednisolone dose was >1 mg/kg daily. Two of 7 patients who were not receiving corticosteroids initiated methylprednisolone treatment after diagnosis of TAM, concomitant with the dose reduction of tacrolimus.

In 65 episodes, patients were on tacrolimus treatment with a median trough blood level of 9.3 ng/dL (range, 3-24 ng//dL) at the time of diagnosis of TAM. The highest tacrolimus blood level at or within the week before TAM was >15 ng/dL in 26 patients (39.4%). Upon diagnosis of TAM, tacrolimus was temporarily discontinued in 12 (18.5%), switched to cyclosporine in 3 (4.6%), and its dose was reduced in 29 episodes (44.6%). Tacrolimus dosing was adjusted to produce trough levels in the lower portion of the therapeutic range (5-10 ng/mL). The most common reasons for discontinuation were worsening renal function and neurologic manifestations. There was no difference of median and highest trough tacrolimus levels at TAM among cases that tacrolimus was discontinued or continued. Supratherapeutic levels were managed primarily by dose reduction. Glucocorticoids were routinely initiated or increased for prevention of GVHD if tacrolimus was discontinued.

Plasma Exchange

Sixty-six of 69 episodes (95.7%) were treated with PE with a median number of 10 (range, 2-33). In the remaining 3 episodes, patients were considered too unstable to tolerate the procedure. Fourteen complications possibly related to PE were observed in 12 TAM episodes (14.5%), including blood pressure changes (hypertension, n = 3; hypotension, n = 4), hypoxia (n = 2), seizures in the setting of hypertension (n = 3), and volume overload (n = 1). One patient (1.3%) had an intracranial hemorrhage.

Disease Course and Response to Treatment with PE

A response of TAM, as defined above, was observed in 38 of 66 episodes in patients who received

Table 2. *Results of Multivariable Cox Proportional Hazards Models*

Variables	HR	95% CI	P
Female	2.09	1.29-3.41	0.003
Lymphoid malignancy	1.75	1.07-2.87	0.03
MUD	2.41	1.48-3.94	<0.001
aGVHD*	4.33	2.63-7.15	<0.001

HR indicates hazard ratio; CI, confidence interval; MUD, matched unrelated donor.
*Time-dependent variable.

Table 3. *Clinical Features of TAM Episodes at Diagnosis*

Variables	Baseline Values before Onset of TAM			Values at TAM Diagnosis		
	No.	Median	25%-75% Percentile	No.	Median	25%-75% Percentile
Platelet count ($\times 10^9$/L)	69	42	23-98	69	19	15-25
Hemoglobin (g/dL)	69	9.1	8.4-10.2	69	8.5	7.8-9.3
LDH (IU/L)	69	964	699-1331	69	2807	2329-4514
Creatinine (mg/dL)	69	1.1	0.9-1.5	69	1.4	1.1-1.9
Total bilirubin (mg/dL)	69	0.7	0.1-1.0	69	1.6	1.1-2.6
Indirect bilirubin (mg/dL)	10	0.7	0.3-1.1	51	1.1	0.7-1.5

PE with a cumulative incidence of response of 60%. Hypertension usually responded promptly. Median time to response was 29 d. We analyzed the association between response and baseline disease characteristics for the first TAM episodes in 63 patients who were treated with PE. Only presence of fungal infection was found to be negatively associated with response on univariate analysis (hazard ratio, 0.3; 95% confidence interval, 0.1-0.9; $P = .03$; Figure 4). Response of TAM was related to control of GVHD and active infections (Table 4). No response of TAM was documented in the absence of response of underlying infections and likewise all but 1 response was observed in patients whose GVHD was controlled with appropriate treatment. The approach to tacrolimus treatment (discontinuation, dose reduction, or no change) in patients with therapeutic blood levels was found to have no affect on TAM response.

Survival and Cause of Death

Fifty-five of 66 patients with TAM died at a median of 3 mo from the date of the first episode. Median survivals from the date of the first episode for nonresponders and responders were 1.07 and 7.5 mo, respectively ($P < .0001$). Survivals at 6 months were 0% and 50.7% for TAM nonresponders and responders, respectively (Figure 5). Despite control of the clinical manifestations in TAM responders, there was a high rate of mortality related to acute or chronic GVHD (with or without complicating infections; Table 5). Four patients with no response of TAM died of hemorrhage, 2 with intracranial bleeding and 2 with diffuse alveolar hemorrhage. One patient with response to therapy died of intracranial bleeding 1 mo after resolution of TAM.

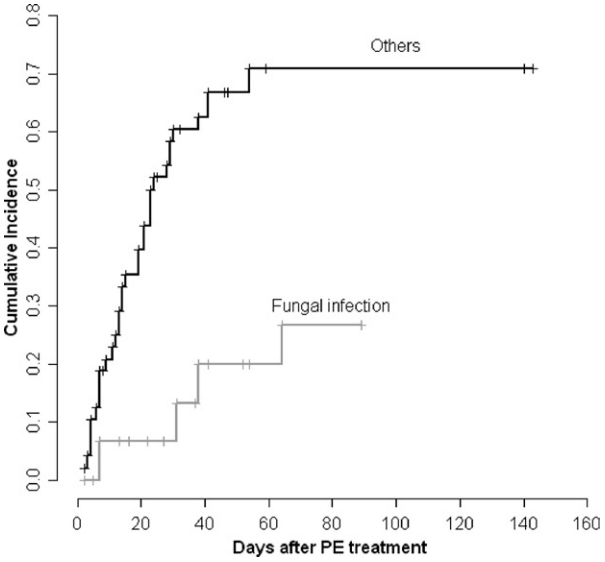

Figure 4. Cumulative incidence of response to TAM was similar for patients with no infection, isolated CMV, and bacterial and/or other viral infections (72%, 70%, and 70%, respectively). Patients with fungal infection had a lower cumulative incidence of response (20%).

DISCUSSION

TAM after transplantation is life-threatening complication of allogeneic SCT. TAM is a complex syndrome that may result from multiple precipitating factors [2]. In this retrospective, single-center cohort of 1219 patients, we used stringent diagnostic criteria based on a consensus formed by an international working group [19]. We included only patients with tacrolimus-based GVHD prophylaxis. Cyclosporine is well established as cytotoxic to endothelial cells in vitro and in vivo, and cyclosporine may cause TAM

Table 4. *TAM Response Related to Response of Coexisting Infection or GVHD*

	Response to Appropriate Treatment		TAM Response	
	No.	%	No.	%
Infection at TAM				
No infection	NA	NA	14/20	70
CMV infection*	10/10	100	7/10	70
Bacterial and/or viral infection†	15/21	71.4	13/21	61.9
Fungal infection	4/15	26.6	4/15	26.6
GVHD status at TAM				
None	NA	NA	10/15	66.73
Quiescent	NA	NA	18/24	75
Active	11/27	40.7	10/27	37

NA indicates not applicable.
*Included patients with isolated CMV infection.
†Includes patients with infections other than isolated CMV and fungal infection.

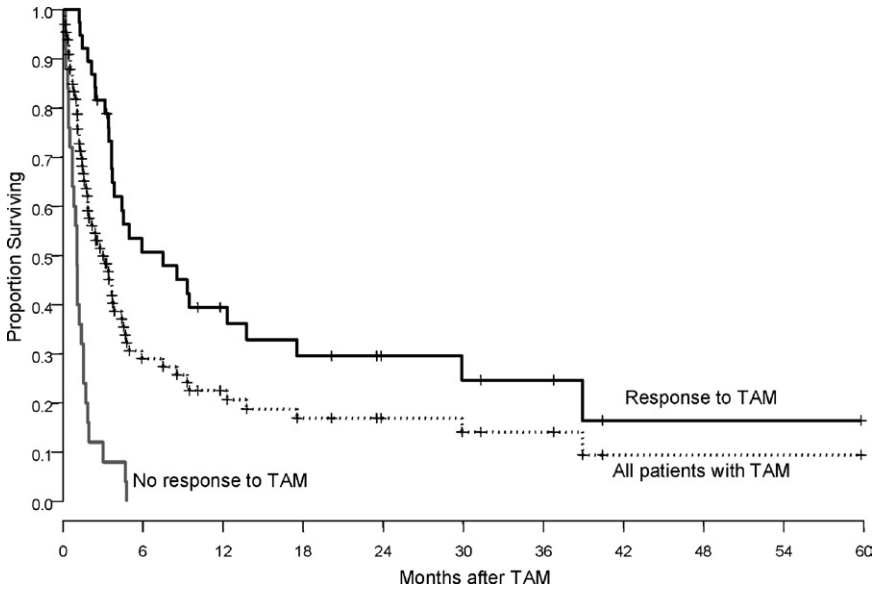

Figure 5. Overall survival after TAM diagnosis was significantly shorter for patients with no response to therapy compared with responders (median overall survival, 1 and 7.5 mo, respectively; $P < .001$).

even without other regimen-related toxicity and/or GVHD [16,21]. The literature of TAM associated with tacrolimus treatment is not as well established. The cumulative incidence of TAM was 5.9% in this study. The question of whether TAM is more or less frequent with tacrolimus compared with cyclosporine requires direct comparison of these agents in comparable patient groups using uniform diagnostic criteria.

In this study, the major risk factors for development of TAM were female sex, diagnosis of a lymphoid malignancy, receipt of an unrelated donor transplant, and development of aGVHD. Patients receiving unrelated donor or mismatched transplants received ATG in the preparative regimen; it is not possible to assess whether treatment with ATGn was independently associated with TAM. The increased risk of TAM with female gender has been reported in the nontransplantation [22,23] and transplantation [9,10,24] settings. To our knowledge, increased risk with a lymphoid malignancy has not been reported. Although the explanation is unknown, these patients were heavily pretreated, and cumulative exposure to chemotherapy and radiation might play a role.

Table 5. *Causes of Death after TAM by Response to Therapy*

Cause of Death	Responders (%)	Nonresponders (%)
aGVHD	3 (11.1)	13 (46.4)
cGVHD	9 (33.3)	4 (14.3)
Infection	1 (3.7)	4 (14.3)
Progression of malignancy	8 (29.7)	1 (3.5)
Hemorrhage	1 (3.7)	4 (14.3)
Other	5 (18.5)	2 (7.2)
Total	27	28

GVHD is an established risk factor for TAM [1,9,24,25]. An association between inflammatory cytokines and thrombosis has been suggested. [26] A recent study reported that IL-8 and TNF-α stimulated ultralarge von Willebrand factorin a dose-dependent manner and IL-6 inhibited ultralarge von Willebrand factor cleavage [27]. Another study reporting endothelial cells as targets of alloreactive donor cytotoxic T lymphocytes has suggested that host endothelium may be another target organ for GVHD, which may explain its association with TMA [28]. We found no response of TAM in the absence of control of GVHD, further supporting a role of GVHD in the pathophysiology.

TAM may occur with reduced intensity preparative regimens [29-32]. We did not observe a difference in the risk of TAM with RIC compared with ablative conditioning. However, most of our patients received fludarabine, which has been reported to cause apoptosis of endothelial cells and to increase allogenicity of endothelial cell targets for $CD8^+$ T cells after HSCT [33]. Direct endothelial toxicity from fludarabine might offset a possible benefit of less intensive regimens.

We did not analyze whether higher blood trough tacrolimus levels were predisposing to development of TAM due to our limited resources to collect 3-4 measurements of blood trough tacrolimus levels before a defined index date for all 1219 patients of this cohort. However, one might assume that our patients with TAM would not have higher levels of tacrolimus because most of the diagnoses were within the first 100 d when the patients were still followed at our institution and their blood trough tacrolimus levels were monitored meticulously to keep it within the therapeutic range.

The management of calcineurin inhibitors (cyclosporine and tacrolimus) poses a therapeutic dilemma in patients with TAM. These agents are important for prevention and treatment of GVHD, which may flare if these agents are discontinued. Many reports have recommended dose reduction or temporary discontinuation of cyclosporine after TAM [9,34]. There are no established guidelines regarding dose adjustment of tacrolimus. In our study, we continued tacrolimus with dose adjustment to the lower therapeutic range, unless progressive renal dysfunction or encephalopathy occurred. Patients with supratherapeutic tacrolimus levels at TAM diagnosis had similar response outcome as patients with therapeutic drug levels. Our results suggest that tacrolimus may be continued after the diagnosis of TAM, particularly if concomitant infections can be controlled.

All patients underwent therapeutic PE unless they were unable to tolerate the procedure. Sixty percent responded with resolution of the signs of microangiopathy, which is comparable to reported results with idiopathic thrombotic thrombocytopenic purpura [22]. This should be interpreted with caution. The use of PE is controversial and assessment of its efficacy in this setting requires a controlled trial. We found PE was generally effective in controlling the symptoms of microangiopathy. However, even with response of TAM, there was a high rate of mortality, mostly secondary to GVHD or progression of the underlying malignancy.

Infections may have an important role in the pathophysiology of TAM after HSCT [24,35]. Transplant recipients typically have multiple episodes of infection, which may include different pathogens, making analysis of the relation complex. In our series, infection was present in 70% of patients at TAM diagnosis. Fungal infection, which has a low response rate to appropriate treatment, was the only negative predictor for response to TAM therapy. There was no documented response of TAM without control of the associated infection and control of GVHD.

In conclusion, this study indicates that post-transplantation TAM is a serious, life-threatening complication after allogeneic HSCT using tacrolimus-based GVHD prophylaxis. The cumulative incidence of response to treatment including PE was 60% and required control of coexisting GVHD and infections. Mortality remains high even in responders, with GVHD or progression of the underlying malignancy as the most common causes of death. This indicates the importance of novel nontoxic strategies for better control of GVHD and infection, which may favorably affect the incidence of TAM and its response to treatment.

REFERENCES

1. Holler E, Kolb HJ, Hiller E, et al. Microangiopathy in patients on cyclosporine prophylaxis who developed acute graft-versus-host disease after HLA-identical bone marrow transplantation. *Blood*. 1989;73:2018-2024.
2. George JN, Li X, McMinn JR, Terrell DR, Vesely SK, Selby GB. Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic HPC transplantation: a diagnostic dilemma. *Transfusion*. 2004;44:294-304.
3. Pettitt AR, Clark RE. Thrombotic microangiopathy following bone marrow transplantation. *Bone Marrow Transplant*. 1994; 14:495-504.
4. Zeigler ZR, Shadduck RK, Nemunaitis J, Andrews DF, Rosenfeld CS. Bone marrow transplant-associated thrombotic microangiopathy: a case series. *Bone Marrow Transplant*. 1995;15: 247-253.
5. Greeno EW, Bach RR, Moldow CF. Apoptosis is associated with increased cell surface tissue factor procoagulant activity. *Lab Invest*. 1996;75:281-289.
6. Bombeli T, Karsan A, Tait JF, Harlan JM. Apoptotic vascular endothelial cells become procoagulant. *Blood*. 1997;89:2429-2442.
7. Bombeli T, Schwartz BR, Harlan JM. Endothelial cells undergoing apoptosis become proadhesive for nonactivated platelets. *Blood*. 1999;93:3831-3838.
8. Kwaan HC, Soff GA. Management of thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *Semin Hematol*. 1997;34:159-166.
9. Ruutu T, Hermans J, Niederwieser D, et al. Thrombotic thrombocytopenic purpura after allogeneic stem cell transplantation: a survey of the European Group for Blood and Marrow Transplantation (EBMT). *Br J Haematol*. 2002;118: 1112-1119.
10. Martinez MT, Bucher C, Stussi G, et al. Transplant-associated microangiopathy (TAM) in recipients of allogeneic hematopoietic stem cell transplants. *Bone Marrow Transplant*. 2005;36: 993-1000.
11. Dlott JS, Danielson CF, Blue-Hnidy DE, McCarthy LJ. Drug-induced thrombotic thrombocytopenic purpura/hemolytic uremic syndrome: a concise review. *Therap Apher Dial*. 2004;8:102-111.
12. Qu L, Kiss JE. Thrombotic microangiopathy in transplantation and malignancy. *Semin Thromb Hemost*. 2005;31:691-699.
13. Furlan M, Robles R, Solenthaler M, Wassmer M, Sandoz P, Lammle B. Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. *Blood*. 1997;89:3097-3103.
14. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med*. 1998;339:1585-1594.
15. van der Plas RM, Schiphorst ME, Huizinga EG, et al. Von Willebrand factor proteolysis is deficient in classic, but not in bone marrow transplantation-associated, thrombotic thrombocytopenic purpura. *Blood*. 1999;93:3798-3802.
16. Zoja C, Furci L, Ghilardi F, Zilio P, Benigni A, Remuzzi G. Cyclosporin-induced endothelial cell injury. *Lab Invest*. 1986; 55:455-462.
17. Ruutu T, Barosi G, Benjamin RJ, et al. Diagnostic criteria for hematopoietic stem cell transplantation-associated microangiopathy (TAM): results of a consensus process by an international working group. *Blood*. 2005;106:400.

18. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
19. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol*. 1991;28:250-259.
20. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
21. Brown Z, Neild GH, Willoughby JJ, Somia NV, Cameron SJ. Increased factor VIII as an index of vascular injury in cyclosporine nephrotoxicity. *Transplantation*. 1986;42:150-153.
22. Rock GA. Management of thrombotic thrombocytopenic purpura. *Br J Haematol*. 2000;109:496-507.
23. Miller DP, Kaye JA, Shea K, et al. Incidence of thrombotic thrombocytopenic purpura/hemolytic uremic syndrome. *Epidemiology*. 2004;15:208-215.
24. Fuge R, Bird JM, Fraser A, et al. The clinical features, risk factors and outcome of thrombotic thrombocytopenic purpura occurring after bone marrow transplantation. *Br J Haematol*. 2001;113:58-64.
25. Paquette RL, Tran L, Landaw EM. Thrombotic microangiopathy following allogeneic bone marrow transplantation is associated with intensive graft-versus-host disease prophylaxis. *Bone Marrow Transplant*. 1998;22:351-357.
26. Seeber C, Hiller E, Holler E, Kolb HJ. Increased levels of tissue plasminogen activator (t-PA) and tissue plasminogen activator inhibitor (PAI) correlate with tumor necrosis factor alpha (TNF alpha)-release in patients suffering from microangiopathy following allogeneic bone marrow transplantation (BMT). *Thromb Res*. 1992;66:373-383.
27. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood*. 2004;104:100-106.
28. Biedermann BC, Sahner S, Gregor M, et al. Endothelial injury mediated by cytotoxic T lymphocytes and loss of microvessels in chronic graft versus host disease. *Lancet*. 2002;359:2078-2083.
29. Shimoni A, Yeshurun M, Hardan I, Avigdor A, Ben-Bassat I, Nagler A. Thrombotic microangiopathy after allogeneic stem cell transplantation in the era of reduced-intensity conditioning: the incidence is not reduced. *Biol Blood Marrow Transplant*. 2004;10:484-493.
30. van Besien K, Bartholomew A, Stock W, et al. Fludarabine-based conditioning for allogeneic transplantation in adults with sickle cell disease. *Bone Marrow Transplant*. 2000;26:445-449.
31. Kornacker M, Luft T, Ho AD, Schaefer HJ. Thrombotic microangiopathy after combined autografting-allografting for multiple myeloma - report of three cases. *Eur J Haematol*. 2005;74:250-253.
32. Corradini P, Tarella C, Olivieri A, et al. Reduced-intensity conditioning followed by allografting of hematopoietic cells can produce clinical and molecular remissions in patients with poor-risk hematologic malignancies. *Blood*. 2002;99:75-82.
33. Eissner G, Multhoff G, Gerbitz A, et al. Fludarabine induces apoptosis, activation, and allogenicity in human endothelial and epithelial cells: protective effect of defibrotide. *Blood*. 2002;100:334-340.
34. Zent R, Katz A, Quaggin S, et al. Thrombotic microangiopathy in renal transplant recipients treated with cyclosporin A. *Clin Nephrol*. 1997;47:181-186.
35. Roy V, Rizvi MA, Vesely SK, George JN. Thrombotic thrombocytopenic purpura-like syndromes following bone marrow transplantation: an analysis of associated conditions and clinical outcomes. *Bone Marrow Transplant*. 2001;27:641-646.